Epigenetic Factors before and during Pregnancy


Abstract

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Endocrine Interactions in the Control of Fetal Growth

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Abstract

Hormones are both growth stimulatory and growth inhibitory in utero. They act as environmental and maturational signals in regulating tissue accretion and differentiation during late gestation. They ensure that fetal development is appropriate for the nutrient supply and is optimal for neonatal survival. Growth-stimulatory hormones, such as insulin, the insulin-like growth factors and the thyroid hormones, have anabolic effects on fetal metabolism and increase cellular nutrient uptake and energy production for tissue accretion. Thyroid hormones also have specific effects on tissue differentiation at key developmental milestones. Similarly, leptin appears to affect development of specific fetal tissues and may counterbalance the maturational actions of other hormones near term. Glucocorticoids inhibit growth in utero but are essential for prepartum tissue differentiation in preparation for delivery. They also affect fetal bioavailability of most of the other growth-regulatory hormones. In addition, many of these hormones alter the placental capacity to supply nutrients for fetal growth. In producing a fetoplacental epigenome specific to the prevailing intrauterine environment, hormones interact to produce phenotypical diversity with potential health consequences long after birth.

Introduction

Hormones are both growth stimulatory and growth inhibitory in utero [1]. They regulate tissue growth and development through actions on cell proliferation and differentiation. They have anabolic and catabolic actions on fetal metabolism and alter the phenotype of the placenta, the principal source of nutrients
for fetal growth [2]. They signal nutrient availability to the fetus and fetal nutrient demands for growth back to the placenta [1, 2]. They also act as maturational signals near term [3]. By modifying the fetal growth trajectory, hormones have a central role in programming development in utero and in ensuring survival both before and at birth [1, 4]. The main growth regulatory hormones during late gestation are insulin, the insulin-like growth factors (IGFs), the thyroid hormones, cortisol and possibly leptin [1]. Of these, some increase in concentration towards term while others maintain stable values throughout late gestation in normal conditions (fig. 1a). This review examines the role of hormones in controlling fetal growth with particular emphasis on the endocrine interactions involved during late gestation.

**Insulin**

Insulin is essential for normal fetal growth [1]. Its deficiency in utero lowers fetal bodyweight but has little apparent effect on placental weight or fetal tissue differentiation (table 1). In fetal sheep, surgical removal of the pancreas reduces fetal growth rate uniformly by 50–60% during late gestation (fig. 1b) and results in a symmetric type of intrauterine growth restriction (IUGR) [8]. These growth defects can be prevented by giving insulin replacement [8]. In contrast, induction of hyperinsulinemia has less consistent effects on fetal growth and is associated primarily with increased adiposity [4]. Weight gain in response to fetal hyperinsulinemia is, therefore, greater in species that have a high fat content at birth [3].

Insulin stimulates fetal growth, in part, by its anabolic actions on glucose and amino acid metabolism [17]. It increases the tissue uptake of these metabolites and enhances the rates of glucose utilization and protein synthesis by fetal sheep [4, 17]. Fetal glucose and amino acid concentrations, therefore, fall in response to fetal insulin administration. This increases the transplacental concentration gradient for glucose and its diffusion into the fetus [2]. Consequently, more glucose and amino acids are available for fetal growth and energy production in the presence of insulin. Although fetal insulin concentrations normally rise in parallel with fetal glucose concentrations, insulin is not primarily a glucoregulatory hormone in utero but, rather, acts to match the fetal rate of glucose utilization to the placental rate of glucose supply [1, 4]. Thus, fetal insulin concentrations are directly related to fetal rates of growth and glucose metabolism [1]. However, these actions of insulin may be mediated, in part, by the IGFs as plasma IGF-1 concentrations are low in pancreatectomized fetuses [6].
Fig. 1. Mean values of plasma concentrations of leptin, cortisol, $T_3$, insulin and IGF-1 in fetal sheep during normal conditions (a), and growth rate (±SEM) measured as crown rump (CRL) increment in control, sham-operated, pancreatectomized, adrenalectomized and cortisol-infused sheep fetuses with respect to days from term (assigned as 140 days of gestation; b). Columns with different letters are significantly different from each other ($p < 0.02$, one-way repeated measures ANOVA). * $p < 0.05$, significantly different from value in control fetuses (two-way repeated-measures ANOVA). Data from references [3–9].
Table 1. Effects of manipulating hormone concentrations on the growth, development and endocrine interactions of fetal sheep during late gestation

<table>
<thead>
<tr>
<th>Hormone</th>
<th>Procedure</th>
<th>Growth (% normal at term)</th>
<th>Specific tissue defects</th>
<th>Endocrine interactions</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>bodyweight</td>
<td>CRL</td>
<td></td>
</tr>
<tr>
<td>Insulin</td>
<td>pancreatectomy administration</td>
<td>70</td>
<td>100–120</td>
<td>90</td>
</tr>
<tr>
<td>IGF-1</td>
<td>administration</td>
<td>100</td>
<td>100</td>
<td>liver, lung, heart, kidney, adrenal</td>
</tr>
<tr>
<td>Thyroid hormones</td>
<td>thyroidectomy</td>
<td>70</td>
<td>90</td>
<td>wool follicles, lungs, skeletal muscle, liver, adrenal gland, bones, heart, SNS, CNS</td>
</tr>
<tr>
<td></td>
<td>T₃ administration</td>
<td>100</td>
<td>100</td>
<td>liver</td>
</tr>
<tr>
<td>Leptin</td>
<td>administration</td>
<td>100</td>
<td>100</td>
<td>liver, adipose tissue</td>
</tr>
<tr>
<td>Cortisol</td>
<td>adrenalectomy</td>
<td>115</td>
<td>110</td>
<td>liver, lungs, gut, pituitary, heart</td>
</tr>
<tr>
<td></td>
<td>administration</td>
<td>80–85</td>
<td>90</td>
<td>liver, lungs, gut, brain, heart, skeletal muscle, placenta</td>
</tr>
</tbody>
</table>

Data from references [4–6, 10–16, 19]. SNS = Sympathetic nervous system; CNS = central nervous system.
Insulin-Like Growth Factors

Like insulin, IGFs stimulate fetal growth [6]. Deletion of either the Igf1 or Igf2 gene in mice reduces fetal bodyweight by 40% at term and leads to developmental abnormalities in a range of fetal tissues [18]. Deletion of both genes or of the Igf type 1 receptor through which the two IGFs act causes an even more severe form of IUGR, which is lethal at birth [18]. Defects in expression of the IGF1 or IGFR1 gene also lead to severe IUGR in human infants [18]. Conversely, fetal overexposure to IGF-2 causes macrosomia and specific organomegaly in mice, cattle, sheep and human infants [2, 6]. In contrast, administration of IGF-1 has little effect on overall growth of normal fetal sheep, although it increases the weight of individual fetal tissues (table 1). However, in growth-restricted fetuses, both body and organ weights are improved by IGF-1 treatment [19].

The IGFs are mitogens which regulate cell proliferation and differentiation [6]. In particular, IGF-2 appears to control the balance between these two processes, especially close to term when fetal tissues are maturing in preparation for birth [1, 6]. IGF-1 also has anabolic effects on fetal metabolism [6, 19]. In fetal sheep, IGF-1 administration stimulates glucose utilization, although to a lesser extent than insulin [19]. It also reduces protein catabolism and oxidation of amino acids in utero with the net effect that fetal protein synthesis increases [6, 19]. In part, these actions of IGFs may be mediated indirectly by changes in placental development [2, 6]. IGF-2 overexpression causes placentomegaly, whereas deletion of the Igf2 gene restricts placental growth, in line with the changes in fetal weight. IGF-2 also influences the transport phenotype of the mouse placenta by altering its morphology and nutrient transporter abundance [18]. Similarly, IGF-1 has been shown to alter nutrient transfer across ovine placenta in vivo and human syncytiotrophoblast in vitro [19, 20].

Tissue expression and circulating concentrations of the IGFs are influenced by a number of nutritional and hormonal factors [6], although basal IGF-1 concentrations vary little during late gestation (fig. 1a). IGF-1 is more responsive to fetal glucose and oxygen levels than IGF-2, which indicates that IGF-2 may be the constitutive drive to fetal mass accumulation while IGF-1 acts as a nutrient sensor like insulin, ensuring that fetal growth is commensurate with the nutrient supply [6]. Expression of both IGFs is sensitive to thyroid and glucocorticoid hormones during late gestation, although the responses are tissue specific and dependent on gestational age (table 1). Indeed, in fetal ovine liver, the normal prepartum decline in IGF2 expression and upregulation of IGF1 transcript abundance depends on the coordinated actions of glucocorticoid and thyroid hormones [4]. In turn, the IGFs affect several other endocrine axes involved in
fetal growth including the pancreatic β-cells and adrenal cortex [1, 3, 6]. For instance, IGF-1 infusion lowers insulin concentrations in fetal sheep, which may explain their lack of bodyweight increment during treatment (table 1).

**Thyroid Hormones**

Before birth, thyroid hormones promote general body growth and the development of specific tissues, such as the brain and skeletal-muscular system [1]. Surgical removal of the fetal thyroid gland causes IUGR in species with little, if any, placental transfer of maternal thyroid hormones, such as sheep and pigs [1]. In fetal sheep, thyroidectomy prevents specific developmental events, such as wool follicle differentiation, at mid-gestation and reduces fetal growth more generally during late gestation with reductions in limb lengths and in body and organ weights by term (table 1). All these growth defects can be ameliorated by thyroxine (T₄) replacement [21]. In human and rodent species that have greater placental permeability to maternal thyroid hormones, deficient fetal T₄ production has less marked effects on fetal bodyweight but still has adverse consequences for brain development, particularly close to term [22, 23].

In thyroidectomized fetal sheep, IUGR is asymmetrical with more pronounced reductions in growth of the appendicular than axial skeleton and changes in metatarsal structure and mechanical properties, indicative of delayed bone development [10, 15]. These changes are also associated with a reduction in the circulating levels of osteocalcin, a marker of osteoblast activity, without any change in the plasma concentrations of total calcium or CTX, a marker of osteoclast activity. Fetal hypothyroidism, therefore, appears to lead to reduced bone deposition rather than any change in bone degradation or calcium homeostasis in utero [15]. Abnormal bone structure is also seen in human neonates with congenital hypothyroidism, despite some transplacental transfer of maternal thyroid hormones [23]. Taken together, these findings suggest that bone development is particularly sensitive to thyroid hormone levels in utero.

In several species, the fetal concentration of tri-iodothyronine (T₃) increases towards term (fig. 1a) due to maturational changes in tissue activity of the deiodinases responsible for converting T₄ to T₃ [24]. In turn, the rise in plasma T₃ initiates differentiation of key fetal tissues essential for neonatal survival (fig. 2). Thyroid hormones are also sensitive to nutrient and oxygen availability and, in association with insulin and IGF-1, have an important role in matching fetal growth to the nutrient supply [1]. Fetal T₄ and T₃ concentrations are suppressed by undernutrition and several other types of experimental IUGR [1, 25]. For example, in rats with uterine artery ligation, bodyweight and
plasma T₄ concentrations are positively correlated in the pups at 20–21 days of gestation and at birth [25]. In addition, thyroid hormone receptor binding in skeletal muscle is reduced in newborn runt compared to normal-sized piglets [26].

Thyroid hormones appear to regulate fetal growth directly through anabolic effects on fetal metabolism [21] and/or indirectly via interactions with other endocrine systems (table 1). They can also affect placental growth or transport function [10, 21]. In fetal sheep, thyroidectomy reduces fetal rates of oxygen consumption and glucose oxidation, which can be restored to normal values by T₄ replacement [21]. Overall, plasma fetal T₄ concentrations correlate with fetal oxygen consumption [21], possibly via changes in sodium-potassium ATPase expression and activity [4]. Thus, less energy will be derived from oxidative metabolism in hypothyroid than euthyroid fetuses, which may constrain growth, particularly of nonessential tissues. Fetal thyroidectomy has also been shown to alter gene expression for the growth hor-

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**Fig. 2.** Schematic diagram showing the effects of hormones on tissue accretion and differentiation in fetal sheep during late gestation and the interactions between hormones in controlling these processes. Tissue accretion decreases while tissue differentiation increases towards birth. Stimulatory effects are shown with light grey arrows, while inhibitory effects are shown with dark grey arrows. Solid arrows indicate known effects, dotted arrow potential effects. Data from references [1–9, 12].
mone receptor (GHR), IGF-1 and IGF-2 in fetal liver and skeletal muscle (table 1), which will have consequences for development of these and other tissues, like the placenta, responsive to circulating IGFs [2, 6].

**Leptin**

Leptin is present in the circulation of human and ovine fetuses from mid-gestation, and the genes for leptin and its receptors are expressed widely in fetoplacental tissues [27, 28]. The role of leptin in the control of fetal growth, however, is controversial. In human neonates, umbilical leptin concentration correlates with several indices of intrauterine growth, such as placental and bodyweights, adiposity and bone mineral content [29]. However, there is little evidence to suggest that leptin deficiency affects birthweight or gross morphology in human and murine neonates [27, 28]. Furthermore, in fetal sheep during late gestation, administration of recombinant ovine leptin has no effect on the fetal growth rate or body or organ weights [11, 14]. In fetal rodents, leptin stimulates proliferation of pancreatic islet cells in vitro and is important for the normal development of neuronal and glial lineage cells in the cerebral cortex [27, 30]. It may also antagonize some of the maturational effects of glucocorticoids in the fetal liver near term [11, 13, 28]. Therefore, leptin may have tissue-specific developmental effects and/or act simply as an endocrine marker of fetal size and energy stores, rather than as a physiological regulator of fetal growth per se.

Leptin produced by the placenta may have an important role in controlling placental growth and function [2, 20, 31]. In pregnant mice heterozygous for a mutation in the leptin receptor, placental leptin concentration is elevated, and this is associated with an increase in fetal weight near term, both in mutant and wild-type pups [32]. In addition, leptin treatment of wild-type pregnant mice decreases placental leptin content and causes reductions in both placental and fetal weights [32]. In vitro studies using human trophoblast cells have shown that leptin stimulates proliferation and inhibits apoptotic processes, while molecular inhibition of placental leptin expression upregulates indicators of apoptosis and causes a reduction in cell division [31]. Leptin also increases amino acid transport in human placental villous fragments in vitro [20].

Leptin synthesis in adipose tissue and circulating concentrations in utero are influenced by hormones known to be involved in the control of fetal growth (table 1). Leptin availability is increased by insulin, thyroid and glucocorticoid hormones (fig. 2). Leptin concentrations, therefore, rise in parallel
with the prepartum cortisol surge in fetal sheep (fig. 1a). In human infants, umbilical leptin concentration is positively associated with insulin and IGF-1 concentrations [29]. Exogenous infusion of leptin in fetal sheep increases IGF type 1 receptor protein levels in perirenal adipose tissue and causes a shift in the relative proportions of unilocular and multilocular cells [11, 14]. However, the extent to which leptin is a marker of the activity of hormones like insulin and the IGFs and/or contributes to their growth-regulatory actions remains unclear.

Glucocorticoids

In several species, glucocorticoid administration to either the mother or fetus leads to IUGR [1, 3]. In sheep, cortisol infusion into preterm fetuses reduces their growth rate by 50% to values similar to those seen in older fetuses closer to term (fig. 1b). Similarly, maternal cortisol infusion for 10 days during late gestation reduces fetal growth rate by 30% in association with decreased fetal body and individual tissue weights [3, 33]. Conversely, preventing the normal prepartum cortisol surge towards term by fetal adrenalectomy prevents the normal prepartum decline in fetal growth rate and increases bodyweight at term (fig. 1b). Glucocorticoids are, therefore, responsible for the natural decrease in growth rate towards term (fig. 1) and probably also contribute to IUGR during adverse conditions which raise fetal glucocorticoid concentrations. In addition, glucocorticoids stimulate the morphological and functional differentiation of a wide range of fetal tissues (table 1). They alter cellular availability of receptors, enzymes, ion channels and transporters, which, in turn, activate many processes that have little or no function prenatally but are vital postnatally [1, 3]. Thus, glucocorticoids switch the fetus from tissue accretion to differentiation both at term and in adverse conditions earlier in gestation.

The growth-inhibitory actions of glucocorticoids are mediated indirectly by the placenta and directly by catabolic effects on fetal metabolism [2, 33]. In all species studied to date, administration of either natural or synthetic glucocorticoids reduces placental weight and compromises placental morphology [33]. These treatments also alter placental transport of glucose and amino acids as well as placental production and metabolism of hormones such as leptin, placental lactogens, eicosanoids and the thyroid hormones [2, 33]. Altogether, these placental changes alter bioavailability of key growth regulatory factors and the nutrient supply for fetal growth. In the fetus, glucocorticoids activate glucogenesis, proteolysis and oxidation of amino acids, which further restricts accretion of new tissue [3, 4]. The actions of glucocorticoids on differentiation
are also direct via changes in gene expression and indirect through alterations in other endocrine systems such as the IGFs, leptin and thyroid hormones (fig. 2). Cortisol downregulates IGF2 gene expression in fetal ovine liver in association with upregulated expression of the GHR and adult transcript of the IGF1 genes [4]. It also activates hepatic and renal deiodinase type 1 which converts T₄ to T₃ and downregulates placental deiodinase type 3 which converts T₄ to biologically inactive reverse T₃ [24]. Indeed, since there appears to be no glucocorticoid response element on the relevant promoter of the ovine IGF2 gene [6], cortisol-induced suppression of fetal hepatic IGF2 may be dependent on the increased hepatic T₃ bioavailability. Thus, near term, glucocorticoids may be the master regulator of an endocrine cascade responsible for coordinating tissue growth and differentiation to maximize the chances of surviving at birth (fig. 2).

Conclusions

Hormones act as environmental and maturational signals in the regulation of tissue accretion and differentiation during late gestation (fig. 2). The different growth stimulatory and inhibitory hormones interact in regulating the fetoplacental transcriptome to ensure that fetal growth and development are matched to the nutrient supply and are optimal for immediate survival. However, by producing an epigenome specific to the prevailing intrauterine environment, hormones can modify the fetal growth trajectory with ensuing phenotypical consequences long after birth.

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Disclosure Statement

The authors have nothing to declare.
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